Int J Clin Exp Med 2011;4(4):258-264
www.ijcem.com /ISSN:1940-5901/IJCEM1107006

Original Article

Reference values for echocardiographic parameters and indexes of left ventricular function in healthy, young adult sheep used in translational research: comparison with standardized values in humans

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Received July 18, 2011; accepted October 9, 2011; Epub October 22, 2011; Published November 30, 2011

Abstract: Ovine models of ischemic heart disease and cardiac failure are increasingly used in translational research. However, reliable extrapolation of the results to the clinical setting requires knowing if ovine normal left ventricular (LV) function is comparable to that of humans. We thus assessed for echocardiographic LV dimensions and indexes in a large normal adult sheep population and compared them with standardized values in normal human adults. Bidimensional and tissue Doppler echocardiograms were performed in 69 young adult Corriedale sheep under light sedation. LV dimensions and indexes of systolic and diastolic function were measured. Absolute and body surface area-normalized values were compared to those for normal adult humans and their statistical distribution was assessed. Normalized dimensions (except for end diastolic diameter) as well as ejection fraction and fractional shortening fell within the ranges established by the American Society of Echocardiography and European Association of Echocardiography for normal adult humans. Normalized end diastolic diameter exceeded the upper normal limit but got close to it when correcting for the higher heart mass/body surface area ratio of sheep with respect to humans. Diastolic parameters also fell within normal human ranges except for a slightly lower mitral deceleration time. All values exhibited a Gaussian distribution. We conclude that echocardiographic parameters of systolic and diastolic LV performance in young adult sheep can be reliably extrapolated to the adult human, thus supporting the use of ovine models of human heart disease in translational research.

Keywords: Echocardiography, sheep, left ventricular function, ovine models of heart disease

Introduction

In important areas of basic and translational cardiovascular research, such as myocardial regeneration and angio-arteriogenesis, large mammalian models of heart diseases are increasingly needed. For studies on neovascular formation, pigs and sheep are suitable species because, as humans, they have a very limited capacity for developing collateral circulation. For studies on myocardial regeneration, however, sheep have an advantage: unlike pigs, whose cardiomyocytes may have up to 32 nuclei [1], ovine cardiac myocytes have only 1 to 4 nuclei [2], thus closer resembling the human cardiomyocyte.

For this and other reasons (docility, slow growth rate, easy housing for protocols requiring long term follow up), ovine models of ischemic heart disease and heart failure are increasingly used [3]. This, in turn, requires knowing normal values for cardiac parameters and indexes.

In the present study, we aimed to establish reference normal values for echocardiographic parameters of left ventricular (LV) dimensions and function in a large population of healthy, young adult Corriedale sheep, assess whether they display a normal distribution, and compare them with standardized values for normal human adults.

Materials and methods

All procedures were approved by the Laboratory Animal Care and Use Committee of the Favaloro University, and performed in accordance with the Guide for Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH publication N° 85-23, revised 1996).

Sixty nine adult male Corriedale sheep weighing 29.5 ± 4.1 Kg (range 21-41 Kg) were studied. On arrival to the animal house, they were deparasited with ivermectine and levamisole and vaccinated against tetanus, anthrax, gas gangrene and clostridial enterotoxemia. Adequate health condition was assessed by professional veterinary staff through clinical examination and laboratory tests. The animals were familiarized with the animal house personnel and laboratory environment for at least one week prior to the studies.

Echocardiography

Under light sedation (diazepam 10 mg, i.v.) and with the animal lying on its right lateral decubitus, bidimensional and pulsed wave Doppler echocardiography were performed (Sonos 5500, Hewlett-Packard, Palo Alto, CA or Philips HD11 XE, Philips ultrasound, Bothell, WA) using 2.5 to 4 MHz transducers. Parasternal long- and short-axis views were recorded. End diastole was defined to occur at the peak R wave of the electrocardiogram, and end systole was defined as the minimum systolic ventricular size, according to the operator.

In all 69 sheep the following dimensions were recorded: LV systolic and diastolic wall thickness (SWTh and DWTh, respectively), LV end diastolic and end systolic diameters (EDD and ESD, respectively) and volumes (EDV and ESV, respectively), by single-plane multiple overlapped disks method). It should be noted that biplane methods could not be applied because in sheep it is not possible to obtain truly orthogonal views from the parasternal window. The following indexes were calculated: LV percent fractional shortening (%FS), LV percent midwall fractional shortening (%FSmid) and LV percent ejection fraction (%EF). In order to establish reference values, all parameters were normalized per m^2 body surface area (BSA). BSA was calculated using the following equation [4]:

$$\text{BSA } (m^2) = 0.84 \times \text{body weight } (Kg)^{0.66}$$

In 56 out of 69 animals, it was feasible to obtain four chamber views. Although in these images the apex appeared foreshortened, the Doppler evaluation of mitral valve inflow and tissue Doppler of the mitral annulus could be reliably assessed, on account that the ultrasonic beam resulted acceptably parallel to the interrogated structures and flows, the angle not exceeding 30°. In these animals, the following diastolic parameters were assessed using pulsed wave Doppler: mitral E and A velocities, mitral E wave deceleration time, isovolumic relaxation time. The E/A ratio was calculated. Using pulsed wave tissue Doppler imaging, e' and a' waves were recorded at lateral mitral annulus level. The E/e'ratio, an index of LV filling pressure [5], was also calculated.

All measurements were carried out according to the guidelines of the American Society of Echocardiography (ASE) and European Association of Echocardiography (EAE) [6, 7]. It should be noted that, given that the aorta emerges medially as compared to the human, the parasternal long axis view does not include the aorta, while the right ventricle is still present in an inferior position because the animal lies on its right lateral decubitus.

Statistical analysis

All values are expressed as mean ± SD. Normal distribution of the values was assessed by the Kolmogorov-Smirnov goodness of fit test using SPSS 15.0 software. The distribution was considered not to be normal if $P<0.05$.

Results

Figure 1 shows bidimensional LV long and short axis views at end systole and end diastole. Images of end-diastolic and end-systolic volume calculation are also shown. **Figure 2** shows transmitral flow pulsed Doppler images, as well as tissue Doppler recordings.

Table 1 lists the values for LV dimensions and systolic indexes and **Table 2** those for diastolic parameters. For LV diameters and volumes the

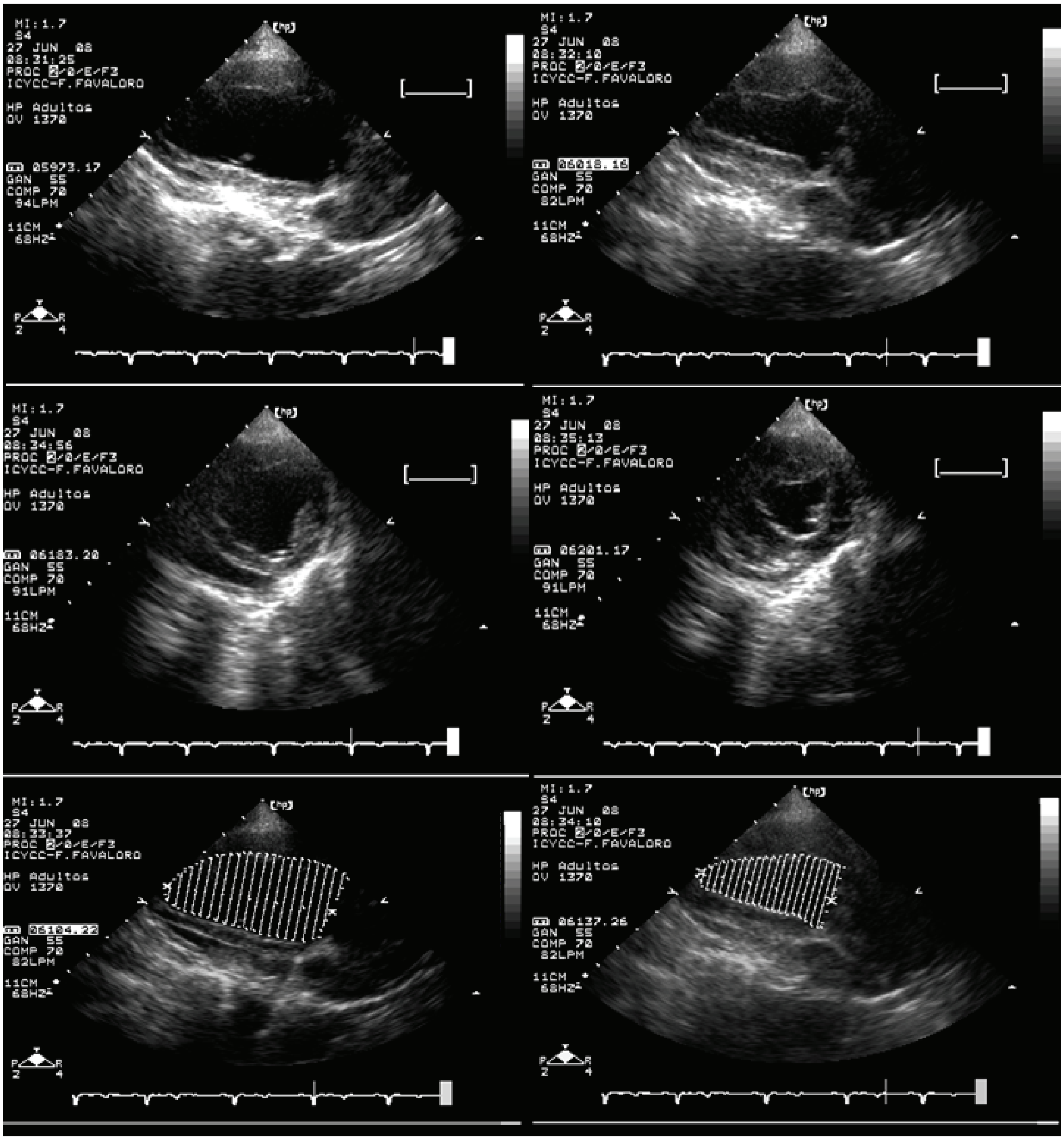

Figure 1. Bidimensional long axis view (upper panel) and short axis view (mid panel) of ovine left ventricle at end diastole (left) and end systole (right). Lower panel: single-plane multiple overlapped disks method for left ventricular volume calculation at end diastole (left) and end systole (right).

absolute and normalized values are shown. BSA was 0.81 ± 0.8 m^2. For all results the distribution was normal, as indicated by consistently non-significant P values. On both tables, reference values according to the guidelines of the ASE and EAE are also listed [6, 7]. Blank spaces correspond to values that, according to the ASE-EAE, either should not be normalized or are not stated in the guidelines. It is seen that, except for normalized end diastolic diameter (see later), all values fit within normal adult human ranges. The values for E and A waves on **Table 2** were taken from Klein et al. [8] as recommended in the guidelines of the ASE-EAE [7].

Discussion

Human cardiac disease is modeled mostly on mice and rats. Although the heart of laboratory rodents share with humans a number of mor-

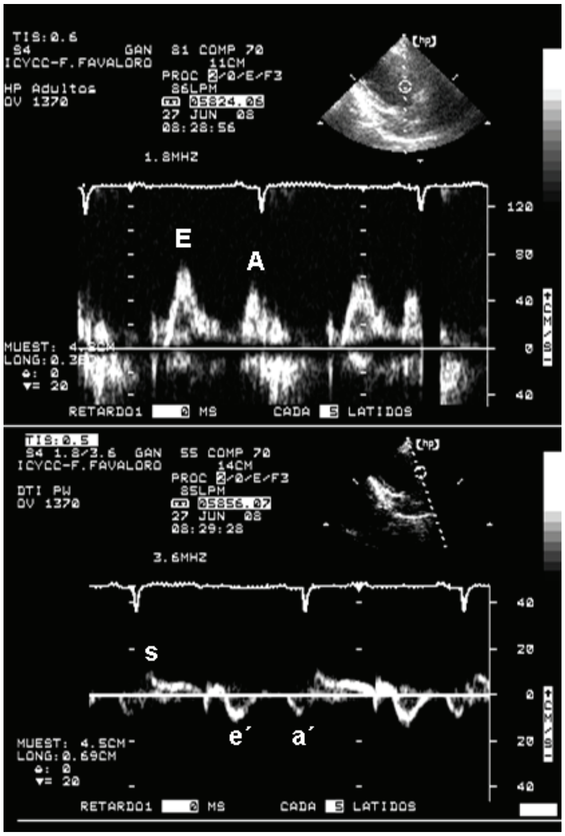

Figure 2. Mitral inflow acquired by pulsed wave Doppler (top) and pulsed tissue Doppler (bottom) recorded from lateral wall-mitral annulus in a healthy sheep. The E, A, s, a' and e' waves are indicated.

phological and physiological features, in terms of variables such as size, gestation time, longevity and cardiomyocyte cell cycle more differences than similarities exist [9]. This may in part explain why results from studies done in small rodents, especially in the field of cardiomyogenesis and angiogenesis, have often failed to be reproduced in humans. In contrast, large mammalian models of cardiac disease may allow a more reliable extrapolation to the clinical setting. At present, pigs and sheep are, in this regard, the preferred species. Because of their easy handling, docility and slow growth rate, sheep are being increasingly used in many laboratories, especially to model acute myocardial infarction and heart failure in studies on heart regeneration, taking advantage of the fact that ovine myocytes possess only one to 4 nuclei, thus resembling the human cardiac muscle cells more than those of the pig. In terms of left ventricular dimensions and function, which constitutes a fundamental end point of this kind of studies, normal reference values obtained from a large animal population are not available. We thus performed echocardiographic assessment of standard LV function parameters in 69 normal, young adult, conscious Corriedale sheep. It should be noticed that the measurements and calculations are based on parasternal views, on account that the ovine thorax morphology (similar to pectus carinatum) provokes apical foreshortening, which difficults obtaining adequate apical, 4-chamber and 2-chamber views for the evaluation of segmental motility and volumes. On the other hand, it was feasible to obtain reliable Doppler measurements. Our results show that the three most widely used indexes of LV systolic performance, namely %EF, %SF and %SFmid, as well as the normalized LV volumes at end diastole and end systole, exhibit mean values well within the ranges established by the ASE and EAE for normal adult humans. In all cases, the measured parameters exhibited a Gaussian distribution.

The echocardiographic values reported by other authors are based on significantly smaller number of sheep. In 8 Dorsett sheep, Moainie et al. reported a LV ejection fraction of 41.2 ± 6.7%, much lower than the 56.7 ± 8.9% of our sheep [10]. This may be due to the fact that Moainie's sheep were under general anesthesia (1% to 2% isoflurane in oxygen), while ours were only slightly sedated. It should be noticed that Moainie et al. performed sub-diaphragmatic echocardiograms requiring laparotomy and calculated LV volumes using the Simpson's rule. In another paper from the same laboratory [11], the baseline ejection fraction calculated from different experimental groups comprising 5 to 10 animals per group ranged from 37.9 ± 2% to 43.7 ± 1.7%. The sedation used was not reported, but given the similarity with the preceding values, it is sound to assume that the sheep were undergoing general anesthesia.

Ejection fraction values closer to those of the present study were reported by Ikeda et al. in 6 sheep (59.4 ± 4.9%) [12], Ghanta et al. in 5 sheep (51.4 ± 12.9%) [13], and ourselves in 7 sheep of another protocol (53 ± 6.2%) [14]. Ikeda's and Ghanta's sheep were under isoflurane anesthesia, while ours were sedated with diazepam as in the present study. Both authors applied the Simpson' rule to work out LV volumes.

Table 1. Ovine and human echocardiographic parameters of LV systolic function

Parameter	Sheep: absolute values	Sheep: values normalized per BSA	Sheep: Gaussian-distribution	Humans: ranges (ASE-EAE)
EDD	35.9 ± 4.3 mm	44.4 ± 4.9 mm/m²	Yes	24-31 mm/m²
ESD	24.1 ± 3.9 mm	29.9 ± 5 mm/m²	Yes	-
DWTh	7.4 ± 0.9 mm	-	Yes	6-9 mm
SWTh	10.5 ± 1.3 mm	-	Yes	-
EDV	45.3 ± 13 ml	55.8 ± 14.3 ml/m²	Yes	35-70 ml/m²
ESV	19.7 ± 7.4 ml	24.1 ± 8 ml/m²	Yes	12-30 ml/m²
%FS	32 ± 8	-	Yes	27-45
%FSmid	19.2 ± 5.9		Yes	14-22
%EF	56.7 ± 8.9	-	Yes	> 55
HR	86 ± 22 b/min			

ASE-EAE: American Society of Echocardiography-European Association of Echocardiography; BSA: body surface area; EDD: end diastolic diameter; ESD: end systolic diameter; DWTh: diastolic wall thickness; SWTh: systolic wall thickness; EDV: end diastolic volume; ESV: end systolic volume; %FS: percent fractional shortening; %FSmid: percent midwall fractional shortening; %EF: percent ejection fraction; HR: heart rate; b: beats.

Table 2. Ovine and human echocardiographic parameters of LV diastolic function

Parameter	Sheep: absolute values	Sheep: Gaussian distribution	Humans: mean ± SD	Humans: range (ASE-EAE)
E wave (cm/s)	53 ± 10	Yes	72 ± 14	-
A wave (cm/s)	39 ± 9	Yes	40 ± 10	-
E/A	1.41 ± 0.38	Yes	1.53 ± 0.40	0.73-2.33
E wave DT (ms)	125.2 ± 36.4	Yes	166 ± 14	138-194
IVRT (ms)	71.4 ± 9.6	Yes	67 ± 8	51-83
E/e’	4.8 ± 1.1	Yes	-	< 8
Lateral e’ (cm/s)	13.7 ± 3.2	Yes	19.8 ± 2.9	14-25.6
Lateral e’/a’	1.7 ± 0.4	Yes	1.9 ± 0.6	0.7-3.1
Lateral E/e’	4 ± 0.9	Yes	-	< 12
HR (beats/min)	81 ± 21			

ASE-EAE: American Society of Echocardiography-European Association of Echocardiography; DT: deceleration time; HR: heart rate; IVRT: isovolumic relaxation time.

Both Borenstein et al. [15] and Psaltis et al. [16] reported LV % fractional shortening rather than % ejection fraction. In both cases, the values for this parameter (38.4 ± 5.7% and 31.6 ± 1.8%, respectively) were similar to ours, despite the fact that in both studies the animals (n=10) were under general anesthesia (isoflurane 2-3%).

With regard to the acquisition window, the subdiaphragmatic approach undoubtedly facilitates obtaining multiple views and better quality images. However, it requires general anesthesia, which affects the measured variables. In addition, its invasive nature (laparotomy needed) severely challenges the possibility of performing serial studies in long term follow up protocols.

It is interesting to note that while ovine normalized end diastolic volume is within the normal human range, normalized end diastolic diameter exceeds by almost 30% the upper limit of the human range. The ratio between left ventricular mass (in g) and body surface area (in m²) is approximately 20% lower in normal adult humans than in 30 Kg weight sheep. By subtracting 20% to the measured value, ovine end diastolic diameter approaches closely to the upper limit of the established human range. For end diastolic and end systolic volumes, the standardized human ranges are considerably larger than for end diastolic diameter (note that there is a 100% variation between the upper and lower limits), thus allowing that our measured volume values fall within the corresponding human ranges, regardless whether or not the correction factor is applied.

With regard to wall thickness, the end diastolic value is within the human normal range. The ASE-EAE guidelines do not report a normal range for end systolic thickness. However, our measured value (10.5 ± 1.3 mm) conveys a systolic wall thickening of approximately 42%, consistent with reported human normal values for bidimensional echocardiography [17].

Diastolic function has been assessed using tissue Doppler imaging by Hashimoto et al. in 8 normal 35-47 kg weight sheep [18]. The reported septal and lateral a' and e' waves and the e'/a' ratio are lower than those of our sheep. However, the different experimental conditions (general anesthesia, open thorax, epicardial recordings) preclude reliable comparisons with the present study.

In our experimental conditions, closely resembling the clinical setting, all diastolic values, except for mitral DT are within the normal human range for the age group 21-40 according to the ASE-EAE [7]. As concerns mitral DT, the mean ovine value was only slightly lower than the lower range limit for the above age group. Therefore, this difference should not challenge the validity of the sheep as a model of human diastolic function.

It should be noticed that displaying similar normal LV function parameters does not anticipate that the sheep model would behave in a similar manner to humans in the setting of disease or physiological perturbations. It just provides a solid ground to propose echocardiographic LV performance variables as reliable end points in this kind of studies.

In conclusion, echocardiographic parameters of systolic and diastolic left ventricular performance in normal, young adult sheep can be reliably extrapolated to the adult human, thus supporting the use of ovine models of human heart disease in translational research.

Acknowledgements

We thank veterinarians María Inés Besansón, Pedro Iguain and Marta Tealdo, and animal house assistants Juan Ocampo, Juan Carlos Mansilla and Osvaldo Sosa for dedicated animal care. The technical help of Julio Martínez and Fabián Gauna is gratefully acknowledged. The authors have no conflicts of interest to disclose. This study was supported by grant PAE-PICT 2007-069 from the National Agency for the Promotion of Science and Technology (ANPCyT) of Argentina.

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References

[1] Gräbner W, Pfitzer P. Number of nuclei in isolated myocardial cells of pigs. Virchows Arch B Cell Pathol 1974; 15: 279-294.

[2] Adler CP, Friedburg H, Herget GW, Neuburger M, Schwalb H. Variability of cardiomyocyte DNA content, ploidy level and nuclear number in mammalian hearts. Virchows Arch 1996; 429: 159-164.

[3] Dixon JA, Spinale FG. Large animal models of heart failure: a critical link in the translation of basic science to clinical practice. Circ Heart Fail 2009; 2: 262-271.

[4] Guyton A, Jones C, Coleman T. Circulatory physiology: cardiac output and its regulation. Philadelphia: WB Saunders Company; 1973; p. 12.

[5] Ommen SR, Nishimura RA, Appleton CP, Miller FA, Oh JK, Redfield MM, Tajik AJ. Clinical utility of Doppler echocardiography and tissue Doppler imaging in the estimation of left ventricular filling pressures: a comparative simultaneous Doppler-catheterization study. Circulation 2000; 102: 1788-1794.

[6] Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise JS, Solomon SD, Spencer KT, Sutton MS, Stewart WJ. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr 2005; 18: 1440-1463.

[7] Nagueh SF, Appleton CP, Gillebert TC, Marino

PN, Oh JK, Smiseth OA, Waggoner AD, Flachskampf FA, Pellikka PA, Evangelista A. Recommendations for the evaluation of left ventricular diastolic function by echocardiography. J Am Soc Echocardiogr 2009; 22: 107-133.

[8] Klein AL, Burstow DJ, Tajik AJ, Zachariah PK, Bailey KR, Seward JB. Effects of age on left ventricular dimensions and filling dynamics in 117 normal persons Mayo Clin Proc 1994; 69: 212-224.

[9] Laguens R, Crottogini A. Cardiac regeneration: the gene therapy approach. Exp Opin Biol Ther 2009; 9: 411-425.

[10] Moainie SL, Gorman JH 3rd, Guy TS, Bowen FW 3rd, Jackson BM, Plappert T, Narula N, St John Sutton MG, Narula J, Edmunds LH Jr, Gorman RC. An ovine model of postinfarction dilated cardiomyopathy. Ann Thorac Surg 2002; 74: 753-760.

[11] Hamamoto H, Gorman JH 3rd, Ryan LP, Hinmon R, Martens TP, Schuster MD, Plappert T, Kiupel M, St John Sutton MG, Itescu S, Gorman RC. Allogeneic mesenchymal precursor cell therapy to limit remodeling after myocardial infarction: the effect of cell dosage. Ann Thorac Surg 2009; 87: 794-801.

[12] Ikeda Y, Yutani C, Huang Y, Masuda K, Yuasa T, Kawaguchi O, Hunyor SN. Histological remodeling in an ovine heart failure model resembles human ischemic cardiomyopathy. Cardiovasc Pathol 2001; 10: 19-27.

[13] Ghanta RK, Rangaraj A, Umakanthan R, Lee L, Laurence RG, Fox JA, Bolman RM 3rd, Cohn LH, Chen FY. Adjustable, physiological ventricular restraint improves left ventricular mechanics and reduces dilatation in an ovine model of chronic heart failure. Circulation 2007; 115: 1201-1210.

[14] Locatelli P, Olea FD, Mendiz O, Salmo F, Fazzi L, Hnatiuk A, Laguens R, Crottogini A. An ovine model of post-infarction dilated cardiomyopathy in animals with highly variable coronary anatomy. ILAR J 2011; 52: e16-21.

[15] Borenstein N, Bruneval P, Behr L, Laborde F, Montarras D, Daurès JP, Derumeaux G, Pouchelon JL, Chetboul V. An ovine model of chronic heart failure: echocardiographic and tissue Doppler imaging characterization. J Card Surg 2006; 21: 50-56.

[16] Psaltis PJ, Carbone A, Nelson A, Lau DH, Manavis J, Finnie J, Teo KS, Mackenzie L, Sanders P, Gronthos S, Zannettino AC, Worthley SG. An ovine model of toxic, nonischemic cardiomyopathy--assessment by cardiac magnetic resonance imaging. J Card Fail 2008; 14: 785-795.

[17] Fineley M, Hickie J. Validity of echocardiographic determination of left ventricular systolic wall thickening. Circulation 1984; 70: 226-232.

[18] Hashimoto I, Bhat AH, Li X, Jones M, Davies CH, Swanson JC, Schindera ST, Sahn DJ. Tissue Doppler-derived myocardial acceleration for evaluation of left ventricular diastolic function. J Am Coll Cardiol 2004; 44: 1459-1466.